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PCT/KR2005/000214

PATENT COOPERATION TREATY

PCT

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference 4FPO-11-16	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. **PCT/KR2005/000214**	International filing date *(day/month/year)* **26 JANUARY 2005 (26.01.2005)**	Priority date *(day/month/year)* 27 JANUARY 2004 (27.01.2004)

International Patent Classification (IPC) or national classification and IPC

C12N 15/00(2006.01)i

Applicant

MOGAM BIOTECHNOLOGY RESEARCH INSTITUTE et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ (sent to the applicant and to the International Bureau) a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
03 AUGUST 2005 (03.08.2005)	28 APRIL 2006 (28.04.2006)
Name and mailing address of the IPEA/KR Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer KIN, Ji Yun Telephone No. 82-42-481-8288

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International application No.
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Box No. I Basis of the report

1. With regard to the **language,** this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1(b))
   ☐ publication of the international application (under Rule 12.4)
   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14.are referred to in this reort as "originally filed" and are not annexed to this report):*

   ☒ the international application as originally filed/furnished

   ☐ the description:
   pages _____ as originally filed/furnished
   pages* _____ received by this Authority on _____
   pages* _____ received by this Authority on _____

   ☐ the claims:
   pages _____ as originally filed/furnished
   pages* _____ as amended (together with any statment) under Article 19
   pages* _____ received by this Authority on _____
   pages* _____ received by this Authority on _____

   ☐ the drawings:
   pages _____ as originally filed/furnished
   pages* _____ received by this Authority on _____
   pages* _____ received by this Authority on _____

   ☒ the sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages _____
   ☐ the claims, Nos. _____
   ☐ the drawings, sheets _____
   ☐ the sequence listing *(specify)* : _____
   ☐ any table(s) related to sequence listing *(specify)* : _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages _____
   ☐ the claims, Nos. _____
   ☐ the drawings, sheets _____
   ☐ the sequence listing *(specify)*: _____
   ☐ any table(s) related to sequence listing *(specify)* : _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-18	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-18	NO
Industrial applicability (IA)	Claims	1-18	YES
	Claims	none	NO

2. Citations and explanations (Rule 70.7)

(1) The following documents have been considered for the purpose of this report;

D1: WO 2001/019868 A1 (MOGAM BIOTECHNOLOGY RESEARCH INSTITUTE) 22 MARCH 2001
D2: Appl. Microbiol. Biotechnol., Vol. 48, pp. 339-345 (1997)
D3: J. Biotechnol., Vol. 85, pp. 41-48 (2001)
D4: Appl. Microbiol. Biotechnol., Vol. 61, pp. 69-76 (2003)

D1 discloses a novel angiogenesis inhibitor, LK8 protein consisting of amino acid sequence of the human apolipoprotein(a) kringle domain V38; a cDNA sequence encoding said LK8 protein; a recombinant expression vector comprising said cDNA; a recombinant microorganism transformed with said expression vector; and a method for producing said LK8 protein.

D2 discloses a delta-integration vector for the insertion of an inducible expression cassette and a bacterial neomycin resistance gene into the genome of *Saccharomyces cerevisiae* via homologous recombination; and a selection of the transformed cell containing integration by resistance to G418.

D3 discloses an overproduction of an anticoagulant hirudin in the delta-integrated recombinant *Saccharomyces cerevisiae* system; and a selection of high copy-number transformants using a dominant selection antibiotic, G418.

D4 discloses a production of cutinase by a recombinant *Saccharomyces cerevisiae* strain induced through the use of a galactose promoter; and a cost effective fermentation strategy for the production of cutinase-batch or fed-batch fermentation.

(2) Novelty

The present invention relates to a recombinant expression vector containing LK8 expression cassette comprising GAL1 promoter, alpha-factor secretion sequence, LK8 cDNA and CYC1 terminator, delta-sequence for the multiple insertion, and a neomycin resistant gene for the selection; a *Saccharomyces cerevisiae* strain transformed with said expression vector; a method for preparing a transformant expressing LK8 protein highly; and a method for mass-production of LK8 protein.

(Continued on Supplemental Sheet.)

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material
      - [x] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [x] on paper
      - [x] in electronic form

   c. time of filing/furnishing
      - [x] contained in the international application as filed
      - [x] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search and/or examination
      - [ ] received by this Authority as an amendment* on _____

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed of furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box No. V.

Since none of the prior art documents disclose a recombinant expression vector comprising LK8 expression cassette, delta-sequence for the multiple insertion of LK8 expression cassette into chromosome of a host strain, and a neomycin resistant gene for the selection, claims 1-18 are considered to be novel under PCT Article 33(2).

(3) Inventive step

Claims 1-5 relate to a recombinant expression vector containing LK8 expression cassette comprising GAL1 promoter, alpha-factor secretion sequence, LK8 cDNA and CYC1 terminator, delta-sequence for the multiple insertion, and a neomycin resistant gene for the selection; and a *Saccharomyces cerevisiae* strain transformed with said expression vector. D1 discloses a cDNA sequence encoding LK8 protein and D2-D3 disclose a delta-sequence for the multiple insertion and a neomycin resistant gene for the selection. It is simple to constitute an expression vector comprising LK8 cDNA known in D1 and the delta-sequence and the neo gene known in D2-D3, and this is obvious to a person skilled in the art. Said recombinant expression vector and said transformed cell have the same function of overproducing a target protein (LK8 protein) as was expected in D1-D3. Therefore, claims 1-5 lack an inventive step as being obvious in view of D1-D3.

Claims 6-10 relate to a method for preparing a transformant expressing LK8 protein highly, comprising i) transformation with the recombinant vector according to claim 1, ii) treatment of G418 and iii) selection of a transformant expressing LK8 protein highly by immunoassay. Since claims 1-5 do not involve an inventive step and the selection of high copy-number transformants using G418 is disclosed in D2-D3, claims 6-10, which involve a method for preparing a transformant expressing LK8 protein highly, lack an inventive step.

Claims 11-18 relate to a method for mass-production of LK8 protein comprising i) transformation with the recombinant vector according to claim 1, ii) seed culture and batch culture of the transformant in a liquid medium containing glucose and galactose, iii) fed-batch culture with galactose, and iv) purification of LK8 protein; and an optimization of culture conditions and purification conditions. Cclaims 1-10 do not involve an inventive step in view of D1-D3 and D4 discloses the cost effective fermentation strategy for the production of target protein (batch or fed-batch fermentation). Therefore, claims 11-18, which involve a method for mass-production of LK8 protein, lack an inventive step. Therefore, claims 1-18 do not appear to involve an inventive step under PCT Article 33(3).

(4) Industrial Applicability

The subject matter of claims 1-18 is considered to be industrially applicable under PCT Article 33(4).